Remarks/Arguments

In view of the above amendments to the claims and the following remarks, reconsideration and allowance of the claims are requested.

Election/Restriction

In response to the election/restriction, applicant withdraws with traverse claims 1-105, 134, 138 and 139. Thus, the election/restriction requirement stands moot. Examiner has also objected to generic nature of claims 130, 131 and 133-142.

Independent claim 130 is directed to a process for enhanced recovery of recombinant insulin, the process comprising treating an expression broth/ culture medium containing the expressing cells with one or more water miscible organic solvents to give a mixture followed by isolating the insulin from the mixture thereof.

Applicant respectfully requests the examiner to reconsider and withdraw the rejections on the presently pending amended claims 130, 131, 133, 135, 136-137 and 140-142 as these claims are directed to a single inventive concept which is to the enhanced recovery of expressed insulin by **treating the culture medium/broth with one or more water miscible solvents**. These water miscible solvents can be selected from one or more of methanol, ethanol, isopropanol, acetic acid, dimethylformamide, dimethylsulfoxide, acetonitrile, dioxan, ethylene glycol, and propylene glycol.

Thus, the applicant affirms the election of single species i.e. water miscible organic solvent as discussed earlier in a telephonic conversation.

The examiner comments that the species listed do not relate to a single general inventive concept. The species lack the same or corresponding special

technical feature for the following reasons: the compounds have different chemical structures and therefore lack unity of invention. The applicants believe that although the elected species i.e. the water miscible solvents, as exemplified above, belong to a diverse class of chemical compound, all these solvents possess a common property, which is their miscibility in water. The miscibility is a term in chemistry that refers to a property of liquids to mix in all proportions, forming a homogeneous solution. All these water miscible solvents when added to water form a homogenous solution, which enhances the extracting property of water, thereby increasing the yield. Table 2 of the present specification (USPN 20060167221) enumerates the following organic solvents specified as water miscible solvents: methanol, ethanol, t-butanol (tertiary-butanol), acetonitrile, dimethylformamide, dimethylsulfoxide, ethyleneglycol, propyleneglycol, and acetic acid. The aforementioned solvents when mixed with water enhance the extraction of insulin polypeptide from the broth. Thus, the overall yield of the insulin polypeptides increases when extracted with one or more water-miscible solvents as compared to the use of water alone. Electing one species from the group of water miscible solvents will unnecessarily limit the scope of invention.

It is therefore evident from the facts and reasoning provided above that the claims have a single general inventive concept i.e. the process for recovering insulin from culture medium/broth using one or more water miscible solvents as claimed in claim 1. Accordingly, reconsideration and withdrawal of the restriction requirement is respectfully requested.

I-Claim rejection - 35 USC § 102

In the most recent Office action, the Examiner has rejected claims 1 and 25 under 35 USC § 102(e) as being anticipated by US 7,091,032 (Annibali).

In view of the present amendment, Applicants respectfully traverse the present rejection and request reconsideration and allowance of the pending claims to the extent that it may be considered that the present rejection is

applicable to the amended claims, for at least one of the following reasons. None of these references teach or suggest each and every limitation recited in the pending claims. Applicants have rewritten the claims to define the invention more particularly and distinctly so as to overcome the technical objections and rejections and define the invention patentably over the prior art.

Claims 1 and 25 have now been canceled and it is believed the present rejection is rendered moot.

As stated above, none of these references relied on by the Examiner teach or suggest each and every limitation recited in the pending claims.

A single prior art reference anticipates a patent claim only if it expressly or inherently describes each and every limitation set forth in the patent claim. Trintec Industries, Inc. v. Top-U.S.A. Corp., 295 F. 3d 1292, 63 USPQ2d 1597 (Fed.Cir.2002); See Verdegaal Bros. v. Union Oil Co. of California, 814 F.2d 628, 631, 2 USPQ2D 1051, 1053 (Fed. Cir. 1987). The identical invention must be shown in as complete detail as is contained in the....claim. Richardson v. Suzuki Motor Co., CO F. 2d 1226, 9 USPQ2d 1913, 1920 (Fed. Cir.1989).

Accordingly, this rejection should be withdrawn.

II. Claim rejections - 35 USC § 103

Rejection based on over US 7,091,032 (Annibali) in view of Willis (Modern Drug Discov., 2001, 4, 43-44)

In the most recent Office action, the Examiner rejected claims 1, 25, 99-101, 130,131, 135, 137-142 under 35 USC § 103(a) as being unpatentable over US 7,091,032 (Annibali) in view of Willis (Modern Drug Discov., 2001, 4, 43-44).

Claims 1, 25, 99-101, 134, 138 and 139 have been canceled and the rejection as to these claims is believed to be rendered moot. As to pending Claims 130, 131, 135, 137 and 140-142 the underpinning facts and reasoning to traverse the obviousness rejection is provided below.

The amended claim 130 is directed to a process of extracting insulin from broth/culture medium containing the expressing cells with one or more water miscible organic solvents to give a mixture, followed by isolating the insulin from the mixture thereof.

The present invention is **directed to the extraction of expressed insulin**. As specified and demonstrated in example 10:
"At the end of fermentation, 1 liter culture broth containing 335 mg of insulin polypeptide in solution was diluted with 20mM citrate buffer, pH3, containing isopropanol at a final concentration of 20%." (See page 7, paragraph 0051).

Thus, the present invention relates to a step which is performed **after** the proteins are expressed in the cell culture, wherein **the expressed protein are extracted and isolated** from the culture medium/broth by treating the culture medium/broth with one or more **water miscible solvents**.

According to the examiner, Annibali teaches a process for the recovery of recombinant insulin comprising treating the culture medium with a water miscible solvent methanol **to induce expression**. As such, the examiner accepts that methanol is used to induce expression. Methanol is **used as a carbon source** by the host *Pichia pastoris*, which has the capability of metabolizing it (See Col. 5, lines 30-21 of Annibali). Further, after the expression of insulin is over and the added methanol is consumed during fermentation as a carbon source, no more methanol is added (See Example 14 of Annibali). During the isolation of insulin from broth culture as described by Annibali is any methanol or any other water miscible solvent added to the culture medium or broth (See Example 13 of Annibali). After completion of the fermentation, cells are directly separated and supernatant is purified using cation exchange chromatography (See Example 14 of Annibali). Annibali, thus, fails to describe or suggest the use of a water miscible solvent or any other solvent for extraction of insulin from the culture

medium or broth. Annibali instead uses a solvent for a different purpose. The amount of alcohol used during fermentation in Annibali is less than 1% (1.2ml/l/h; See Col. 25, lines 20-21 of Annibali), the amount which is generally not used for the extraction purpose.

On the other hand, the present invention discloses a process of extracting insulin from broth/culture medium using one or more water miscible solvents. The process is carried out after the completion of fermentation. Moreover, the amount of water miscible solvent used in the present invention is much higher (10% v/v to 40% v/v) than the amount of methanol used in Annibali (less than 1% v/v).

Thus, Annibali and the present invention (US20060167221) are directed to different aspects of the insulin production. **Annibali is directed to the use of methanol for expression of insulin** (See col. 14; lines 45-47 of Annibali) whereas, the present invention is directed to the use of water miscible solvents to solve the problem associated with the insulin extraction and **not** insulin expression (See page 2, lines 35-41 of US20060167221).

As such, claim 130 is clearly distinguishable from the teaching of Annibali.

The Examiner has taken the position that Annibali teaches a process for the expression of recombinant insulin in *P. Pastoris* comprising treating the culture medium with water miscible solvent. The Examiner has acknowledged that Annibali does not teach the use of expanded-bed chromatography for the isolation of insulin from a broth/culture medium as presently claimed. However, the Examiner cites Willis which is said to teach that expanded bed chromatography for purification of recombinantly expressed protein in cells are known. The Examiner concludes that It would have been obvious to one of skill in the art to substitute expanded-bed chromatography taught by Willis for the traditional chromatography in the method taught by Annibali.

However, as pointed out above, Annibali does not teach the extraction of insulin from the culture medium/broth using one or more water miscible solvent. Nor does it suggest or teach the use of expanded bed chromatography for purification. Willis does not provide that which is missing from the Annibali reference as compared to the presently claimed process.

Willis is a general disclosure of technology and mechanics involved in expanded bed adsorption chromatography (EBA) and its application. There is no disclosure of insulin in Willis. It neither discloses nor teaches the use of any water miscible solvent for increasing the recovery of any peptide or protein from the broth culture. Willis' general disclosure, however, does not describe or suggest the use of such methods for extraction of insulin or any protein form the culture broth as claimed in claim 130. Further, even if there was a suggestion to extract insulin, Willis does not enable such a process.

The examiner relies on two references Annibali and Willis for supporting the rejection on obviousness. Annibali does not teach the use of a water miscible solvent for enhancing the recovery of insulin from culture medium/broth. Willis does not teach or suggest about the recovery of any polypeptide from the culture medium/broth using a water miscible solvent. Applicant submits that it cannot be fairly suggested that one of skill in the art reading Willis would be motivated to combine it with Annibali in the manner purported by the examiner.

Further it is clear that "[t] he mere fact that a device or process utilizes a known scientific principle does not alone make that device or process obvious." (*Uniroyal, Inc. v. Rudkin-Wiley Corp.*, 837 F.2d 1044, 1053, 5 USPQ2d 1434, 1440 (Fed. Cir. 1988)). Mere fact that the expanded bed chromatographic method is a known scientific principle does not make the use of it in a particular process obvious within the meaning of 35 U.S.C. 103 merely because it exists. There must be more to support a prima facie case of obviousness.

Amendment made in claim 130 further obliterates the obviousness rejection. For these reasons, claim 130 and dependent claims 131-133, 135-137 and 140-142 are reasonable deemed patentable over Annibali even taken in view of Willis.

III. Rejection based on USPN 7,091,032 (Annibali) in view of Willis (Modern Drug Discov., 2001, 4, 43-44) in further view of Trinh et al. (Bioseparation, 2000, 9, 223-30 and Scopes et al. (Protein Purification: principles and Practice, Springer, new York, 1994, pp 157-71).

The Examiner has, also, rejected claims 1, 25, 99-101, 130,131, 135, 137-142 under 35 USC § 103(a) as being unpatentable over USPN 7,091,032 (Annibali) in view of Willis (Modern Drug Discov., 2001, 4, 43-44) in further view of Trinh et al. (Bioseparation, 2000, 9, 223-30 and Scopes et al. (Protein Purification: principles and Practice, Springer, new York, 1994, pp 157-71).

Claims 1, 25, 99-101, 138 and 139 have been canceled and as such, the rejection is believed to be rendered moot.

The amended claim 130 is directed to a process of extracting insulin from broth/culture medium with one or more water miscible organic solvents to give a mixture, followed by isolating the insulin from the mixture thereof.

As noted above, Annibali teaches the use of methanol as a carbon source to induce the expression in *Pichia pastoris*. Annibali does not teach the use of a water miscible solvent for increasing the extraction of bound insulin. Willis is a general disclosure of expanded bed adsorption chromatography (EBA).

Trinh et al. discloses a method of purifying bioactive endostatin from *Pichia pastoris* using streamline-SP cation exchange resin as an expanded bed chromatography step. Trinh et al. does not disclose the use of a water-miscible

solvent to increase the recovery of any recombinant protein. Further, Trinh et al. does not teach, motivate or suggest using the expanded bed chromatography or water miscible solvents for insulin isolation or purification. Trinh's general disclosure, however, does not describe or suggest the use of such methods for extraction of insulin or any protein form the culture broth as claimed in claim 130. Further, even if there was a suggestion to extract insulin, Trinh does not enable such a process.

Scopes et al. teaches general methods for ion exchange chromatography including selection of buffers, loading, washing and eluting conditions. Scopes et al. is a generic disclosure. Scopes' general disclosure, however, does not describe or suggest the use of such methods for extraction of insulin or any protein form the culture broth as claimed in claim 130. Further, even if there was a suggestion to extract insulin, Scopes does not enable such a process.

For obviousness rejection, the examiner relies on Annibali, Willis, Trinh et al. and Scopes. Annibali does not teach a method for enhancing the recovery of insulin by treating the broth with water miscible solvent. Willis, Trinh et al. and Scopes do not explicitly or implicitly teach the use of expanded bed chromatography method for purification of insulin which is extracted using a water miscible solvent from a culture medium/broth. Applicant submits that it cannot be fairly suggested that one of skill in the art reading Annibali would be motivated to combine it with Willis, Trinh et al. and Scopes in the manner purported by the examiner.

The only thing that can possibly be read to suggest such a combination of teachings is the disclosure of applicant's invention in the present application.

Yet, even if that were permissible, the combination fails to account for that which is presently claimed. At best, the references relied upon in this rejection are directed to general teachings which fail to address the specific method of isolation of insulin that is actually being claimed. As such it is not reasonable to

conclude that the presently claimed invention would have been obvious over this combination of teachings.

The newly presented amendments made in claim 130 further serves to distinguish the present method from that described in the references relied upon. Thus, applicant requests reconsideration of the present rejection over Annibali in view of Willis, Trinh et al. and Scopes.

IV. Rejection based on USPN 7,091,032 (Annibali) in view of Willis (Modern Drug Discov., 2001, 4, 43-44) in further view of Scopes et al. (Protein Purification: principles and Practice, Springer, new York, 1994, pp 157-71) and Gerlough & Bates (J. Pharm. Exp. Therapeutics, 1932, Vol. XLV, No. 1, pp. 19-51).

In the most recent Office action the Examiner has, also, rejected claims 1, 3, 19-36, 79-83, 88-97, 99-101, 130-135, 137-142 under 35 USC § 103(a) as being unpatentable over US 7,091,032 (Annibali) in view of Willis (Modern Drug Discov., 2001, 4, 43-44) as applied to claims 1, 25, 99-101, 130,131, 135, 137-142 above in further view of Scopes et al. (Protein Purification: principles and Practice, Springer, new York, 1994, pp 157-71) and Gerlough & Bates (J. Pharm. Exp. Therapeutics, 1932, Vol. XLV, No. 1, pp. 19-51).

Claims 1, 3, 19-36, 79-83, 88-97, 99-101, 134, 138, and 139 have been canceled by this response. As such, the rejection is believed to have been rendered moot as to these claims.

The amended claim 130 is directed towards a process of extracting insulin from broth/culture medium containing the expressing cells with one or more water miscible organic solvents to give a mixture, followed by isolating the insulin from the mixture thereof.

Applicant notes the arguments presented above which address the application of the teachings of Annibali, Willis and Scopes et al. to the presently claimed invention.

According to the Office Action, Gerlough and Bates teach a method of purifying insulin comprising alcohol (more than 60%) precipitation of insulin obtained from minced beef pancreas. Scopes teaches that the method of protein precipitation by water miscible solvents has been used since early days of protein purification.

Gerlough and Bates teach purification by fractional precipitation of insulin with alcohol. This process taught by Gerlough and Bates is entirely different form the process claimed in the present invention. The alcohol/water miscible solvent used in the process of present invention is not intended for the precipitation of protein for purification. The water miscible solvent is used to extract the organelle bound insulin in a homogenous solution (composed of buffer and water miscible solvent). The amount of water miscible solvent varies from about 10% to about 40%, which is less than the amount of alcohol used for precipitation of insulin (more than 50%). In fact, Gerlough and Bates teach away from the use of water miscible solvent for extraction of insulin from culture medium/broth as is claimed in claim 130 because the precipitation and extraction are opposite processes.

As noted above, Annibali teaches the use of methanol as a carbon source to induce the expression in *Pichia pastoris*. Annibali does not teach the use of a water miscible solvent for increasing the extraction of bound insulin. Willis and Trinh et al. disclose use of expanded bed chromatography for protein purification. Willis and Trinh et al. do not teach use of water miscible solvents for recovery of any protein. Scopes is a generic disclosure. Gerlough and Bates do not teach a process for enhancing the recovery of insulin by treating the culture medium/broth using water miscible solvent without precipitating the protein. Applicant submits that it cannot be fairly suggested that one of skill in the art reading Annibali would be motivated to combine it with Willis, Trinh et al. Scopes and Gerlough and Bates in the manner purported by the examiner. For these

reasons, claim 130 and dependent claims 131-133, 135-137 and 140-142 are deemed patentable over Annibali in view of Willis, Trinh et al. Scopes and Gerlough and Bates.

Applicant respectfully requests the examiner to reconsider and withdraw this rejection and submits that the claim 130 and dependent claims 131-133, 135-137, 140-142 are allowable over the cited references.

As noted above that the Office Action fails to specifically address even the expressly recited features of the pending independent and dependent claims. Under the Office's policy of compact prosecution, each claim should be reviewed for compliance with every statutory requirement for patentability in the initial review of the application. (MPEP §707.07(g)). Thus, it is submitted that the Office's failure constitutes a failure to expeditiously provide the information necessary to resolve issues related to patentability that prevents the Applicant from, for example, presenting appropriate patentability arguments and/or rebuttal evidence. (See The Official Gazette Notice of November 7, 2003). Additionally, it is submitted that the Office's failure needlessly encourages piecemeal prosecution, which is to be avoided as much as possible. (MPEP §707.07(g)). Accordingly, in the event that the Examiner maintains the rejection of any of the independent and/or dependent claims, Applicant respectfully requests, in the interests of compact prosecution, that the Examiner apply art against each feature of each rejected independent and dependent claims, on the record, and with specificity sufficient to support a prima facie case of obviousness.

The mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. *In re Mills*, 916 F.2d 680, 16 USPQ2d 1430 (Fed. Cir. 1990).

It is well known that in order for any prior-art references themselves to be validly combined for use in a prior-art § 103 rejection, *the references themselves*

(or some otherprior art) must suggest that they be combined. E.g., as was stated in In re Sernaker, 217 U.S.P.Q. 1, 6 (C.A.F.C. 1983):

"[P]rior art references in combination do not make an invention obvious unless something in the prior art references would suggest the advantages to be derived from combining their teachings." That the suggestion to combine the references should not come from applicant was forcefully stated in Orthopedic Equipment Co. v. United States , 217 U.S.P.Q. 193, 199 (C.A.F.C. 1983):

"It is wrong to use the patent in suit [here the patent application] as a guide through the maze of prior art references, combining the right references in the right way to achieve the result of the claims in suit [here the claims pending]. Monday morning quarterbacking is quite improper when resolving the question of nonobviousness in a court of law [here

the PTO]." As was further stated in Uniroyal, Inc. v. Rudkin-Wiley Corp., 5 U.S.P.Q.2d 1434 (C.A.F.C. 1988), "[w]here prior-art references require selective combination by the court to render obvious a subsequent invention, there must be some reason for the combination other than the hindsight gleaned from the invention itself

... *Something in the prior art must suggest the desirability and thus the obviousness of making the combination.*" [Emphasis supplied.]

In line with these decisions, the Board stated in Ex parte Levengood, 28 U.S.P.Q.2d 1300 (P.T.O.B.A.&I. 1993):

"In order to establish a *prima facie* case of obviousness, it is necessary for the examiner to present *evidence*, preferably in the form of some teaching, suggestion, incentive or inference in the applied prior art, or in the form of generally available knowledge, that one having ordinary skill in the art *would have been led* to combine the relevant teachings of the, applied references in the proposed manner to arrive at the claimed invention. ...

That which is within the capabilities of one skilled in the art is not synonymous with obviousness. ... That one can *reconstruct* and/or explain the theoretical mechanism of an invention by means of logic and sound scientific reasoning does not afford the basis for an obviousness conclusion unless that

logic and reasoning also supplies sufficient impetus to have led one of the ordinary skill in the art to combine the teachings of the references to make the claimed invention.... Our reviewing courts have often advised the Patent and Trademark Office that it can satisfy the burden of establishing a *prima facie* case of obviousness only by showing some objective teaching in either the prior art, or knowledge generally available to one of ordinary skill in the art, that 'would lead' that individual 'to combine the relevant teachings of the references.' ... Accordingly, an examiner cannot establish obviousness by locating references which describe various aspects of a patent applicant's invention without also providing evidence of the motivating force which would impel one skilled in the art to do what the patent applicant has done."

In the present case, there is no reason given in the last Office action to support the proposed combination. However the fact that both references teach one or more limitations is not sufficient to gratuitously and selectively suggest that the one would be led to substitute parts of one reference for a part of another reference in order to meet applicants' novel claimed combination.

The references relied upon fail to provide an adequate basis in evidence to support the Examiner's initial conclusion of obviousness. In short there must be more than merely establishing that the individual components exist in the prior art. There must be something , found in the prior art which would have suggested , led or motivated one skilled in this art to bring those individual components together in the manner presently claimed. The present rejection lacks this aspect.

It is respectfully requested that these rejection be reconsider and withdrawn.

Conclusion

Applicants respectfully submit that the patent application is in condition for allowance and notification to that effect is earnestly requested. If desired, the examiner is invited to conduct a telephone conference to expedite the prosecution of the subject application. In such a case, the examiner is invited to call the undersigned attorney.

Should any official at the United States Patent and Trademark Office deem that any further action by the Applicants or Applicants' undersigned representative is desirable and/or necessary, the official is invited to telephone the undersigned at the number set forth below.

The Commissioner is herby authorized to charge any fees which may be required regarding this application under 37 CFR §§ 1.16-1.17 or credit any overpayment, to deposit account No. 503321. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, or otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 503321.

Respectfully submitted,

By: Sam Zaghmout

O. M. (Sam) Zaghmout Ph.D
(Registration No. 51,286)

Contact Information:
Bio Intellectual Property Service (BIO IPS) LLC
8509 Kernon Ct, Lorton, VA 22079. USA
Cell Phone (703-919-4348), Fax: (703-550-0409), (703) 550-1968 (Voice/Fax)